

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH [REDACTED] SERVICE
OFFICE OF EXPERIMENT STATIONS
MAYAGUEZ, PUERTO RICO

FEDERAL EXPERIMENT STATION
IN PUERTO RICO

July 7, 1954

Professor Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison 6, Wisconsin

Dear Professor Lederberg:

Several weeks ago I wrote you that we would attempt to isolate a sample of indican for you. Since then we have tried several times, but have failed to isolate indican from any of the species we have here. We have followed the instructions of published methods on the following

1. Indigofera endecaphylla
2. " subulata
3. " tinctoria

and have been unable to isolate indican.

The following suggestions and comments might be worth considering:

Difficulties are often encountered in the isolation of natural products, even though detailed procedures are at hand. This is especially so in the case of many glycosides. It is possible that we have failed because we did not carry out instructions exactly as described, although we tried to, and were always aware of the difficulties that are encountered in the isolation of glycosides. We are enclosing a copy of the procedure we used. If you wish we will send you samples of the Indigoferas we have here. Since the procedure for isolating indican is "simple" and requires very little time, it is very likely that the professor there at Wisconsin who offers a course in "Organic Preparations" would be more than pleased to carry out the isolation of indican for you. This would lead you a quick source of indican in the event there is indican present in the Indigoferas we have here.

2 - Professor Joshua Lederberg - July 7, 1954

Since we have available a sample of Indigofera tinctoria, we are forwarding it to you by parcel post, hoping that you can use it as suggested above.

Yours sincerely,

Murrell P. Morris

Murrell P. Morris
Chemist

Enclosure

THE ISOLATION OF INDICAN

Perkin and Bloxam

J. Chem. Soc. 91, 1715-1728 (1907)

Page #1718

"and the following simple method originated.

One thousand grams of the leaves and stems of the Indigofera sumatrana (analysed previously and estimated to yield 3.13 per cent. of indigotin) were treated in a large bottle with 4 litres of acetone, the mixture being occasionally shaken during seven days. Up to the present, this operation has always been carried out in the cold, for although this is probably not an essential feature for success, it proceeds so satisfactorily that it has been adopted throughout.

The green-coloured acetone solution was then filtered, the residual leaf rinsed with the solvent, and the liquid evaporated in the first operations by means of a vacuum at the ordinary temperature to a volume of about 150 c.c. In subsequent experiments, however, the acetone was removed by distillation on the steam-bath in the usual manner. To this residue about ten times its volume of light petroleum was added, causing the deposition of a yellowish-brown, viscous precipitate, which was repeatedly agitated with small quantities of light petroleum until a green-coloured extract was no longer formed. The product, on treatment with water, yielded, in the earlier experiments, a pale yellow liquid containing some quantity of grey matter in suspension, which could readily be removed by filtration, but with the more recent samples of plant the precipitate at this stage was too viscous to

permit of separating the solid matter in this way. In such cases, decantation was resorted to, and the solution was clarified by agitation with ether, as but little indican is dissolved by this solvent. The clear aqueous liquid (A), after removal of dissolved ether under reduced pressure, was decanted from a small quantity of tarry deposit, treated with 10 cc.* of N/2 sodium carbonate, to neutralize plant acids, and placed in a vacuum desiccator over sodium hydroxide. Within a few hours the sides of the containing vessel became coated with crystals, and in about three days a semisolid, crystalline mass was obtained. The product was collected on a Buchner funnel and drained on porous tile; when dry, it weighed 13.40 grams. The mother liquor, again evaporated in a similar manner gave a further 6.415 grams of the glucoside.

The residual liquid was still rich in indican, but on concentration was too viscid for filtration, and it is here that a somewhat serious loss of substance occurs. To obviate this as far as possible, the mixture, dissolved in water, was treated with finely-powdered potassium sulphate, which caused the precipitation of a brown, tarry impurity, together with some quantity of the glucoside. The solution was evaporated to dryness in a vacuum, the residue extracted with acetone, the solvent removed from the extract, and the crude indican crystallised from water. In this way, approximately 2 grams of the substance were recovered.

The residual leaf was again submitted to two extractions with acetone, by which means respectively 3.654 and 3.17 grams of indican were obtained. Accordingly, 1000 grams of leaf yielded 31.66 grams of this substance, and this quantity could have been enhanced by a further digestion of the leaf material, preferably with boiling acetone. Analysis showed, for instance, that it still contained 1.3 per cent. of glucoside, but as the object of the work was the rapid preparation of a large quantity of indican, this residue was not again extracted.

* The amount of alkali necessary varies with the sample of plant employed. It is very important that sufficient be added to prevent formation of indigotin, but excess, on the other hand, causes gelatinisation.